

Amendment to the Specification:

- Please delete paragraph [0084] and replace it with the following paragraph:

[0084] In preferred embodiments the targeting element binds to an epitope on pIgR or the pIgR stalk that comprises an amino acid sequence selected from the following: LRKED (SEQ ID NO: 37), QLFVNNEE (SEQ ID NO: 38), LNQLT (SEQ ID NO: 39), YWCKW (SEQ ID NO: 40), GWYWC (SEQ ID NO: 41), STLVPL (SEQ ID NO: 42), SYRTD (SEQ ID NO: 43), QDPRLF (SEQ ID NO: 44) and KRSSK (SEQ ID NO: 45). In more preferred embodiments the targeting element binds to pIgR or the pIgR stalk in a region selected from the following:

- R1 KRSSK (SEQ ID NO: 45) to the carboxy terminus of pIgR;
- R2a From SYRTD (SEQ ID NO: 43) to the carboxy terminus of pIgR,
- R2b From SYRTD (SEQ ID NO: 43) to KRSSK (SEQ ID NO: 45),
- R3a From STLVPL (SEQ ID NO: 42) to the carboxy terminus of pIgR,
- R3b From STLVPL (SEQ ID NO: 42) to KRSSK (SEQ ID NO: 45),
- R3c From STLVPL (SEQ ID NO: 42) to SYRTD (SEQ ID NO: 43),
- R4a From GWYWC (SEQ ID NO: 41) to the carboxy terminus of pIgR,
- R4b From GWYWC (SEQ ID NO: 41) to KRSSK (SEQ ID NO: 45),
- R4c From GWYWC (SEQ ID NO: 41) to SYRTD (SEQ ID NO: 43),
- R4d From GWYWC (SEQ ID NO: 41) to STLVPL (SEQ ID NO: 42),
- R5a From YWCKW (SEQ ID NO: 40) to the carboxy terminus of pIgR,
- R5b From YWCKW (SEQ ID NO: 40) to KRSSK (SEQ ID NO: 45),

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- R5c From YWCKW (SEQ ID NO: 40) to SYRTD (SEQ ID NO: 43),
- R5d From YWCKW (SEQ ID NO: 40) to STLVPL (SEQ ID NO: 42),
- R5e From YWCKW (SEQ ID NO: 40) to GWYWC (SEQ ID NO: 41),
- R6a From LNQLT (SEQ ID NO: 39) to the carboxy terminus of pIgR,
- R6b From LNQLT (SEQ ID NO: 39) to KRSSK (SEQ ID NO: 45),
- R6c From LNQLT (SEQ ID NO: 39) to SYRTD (SEQ ID NO: 43),
- R6d From LNQLT (SEQ ID NO: 39) to STLVPL (SEQ ID NO: 42),
- R6e From LNQLT (SEQ ID NO: 39) to GWYWC (SEQ ID NO: 41),
- R6f From LNQLT (SEQ ID NO: 39) to YWCKW (SEQ ID NO: 40),
- R7a From QLFVNNEE (SEQ ID NO: 38) to the carboxy terminus of pIgR,
- R7b From QLFVNNEE (SEQ ID NO: 38) to KRSSK (SEQ ID NO: 45),
- R7c From QLFVNNEE (SEQ ID NO: 38) to SYRTD (SEQ ID NO: 43),
- R7d From QLFVNNEE (SEQ ID NO: 38) to STLVPL (SEQ ID NO: 42),
- R7e From QLFVNNEE (SEQ ID NO: 38) to GWYWC (SEQ ID NO: 41),
- R7f From QLFVNNEE (SEQ ID NO: 38) to YWCKW (SEQ ID NO: 40),
- R7g From QLFVNNEE (SEQ ID NO: 38) to LNQLT (SEQ ID NO: 39),
- R8a From LRKED (SEQ ID NO: 37) to the carboxy terminus of pIgR,
- R8b From LRKED (SEQ ID NO: 37) to KRSSK (SEQ ID NO: 45),
- R8c From LRKED (SEQ ID NO: 37) to SYRTD (SEQ ID NO: 43),

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R8d From LRKED (SEQ ID NO: 37) to STLVPL (SEQ ID NO: 42),

R8e From LRKED (SEQ ID NO: 37) to GWYWC (SEQ ID NO: 41),

R8f From LRKED (SEQ ID NO: 37) to YWCKW (SEQ ID NO: 40),

R8g From LRKED (SEQ ID NO: 37) to LNQLT (SEQ ID NO: 39), and

R8h From LRKED (SEQ ID NO: 37) to QLFVNNEE. (SEQ ID NO: 38)

- Please delete paragraph [0090] and replace it with the following paragraph:

[0090] Figure 1 provides a schematic illustration of an sFv domain structure, and a model of the interactions between sFvs forming a dimeric “diabody” structure. Peptide fragments shown in SEQ ID NOS 46, 49 & 52.

- Please delete paragraph [0094] and replace it with the following paragraph:

[0094] Figure 5 depicts the coding sequence (SEQ ID NO: 53) of an exemplary pIgR-directed sFv (APL10).

- Please delete paragraph [0095] and replace it with the following paragraph:

[0095] Figure 6 depicts the coding sequence (SEQ ID NO: 54) of an exemplary pIgR-directed sFv-IL-2 fusion protein.

- Please delete paragraph [0096] and replace it with the following paragraph:

[0096] Figure 7 provides maps of exemplary IL-2-sFv expression constructs. Peptide fragments shown in SEQ ID NOS 50 & 51.

- Please delete paragraph [0196] and replace it with the following paragraph:

[0196] Various amino acid sequences are known that may serve as suitable spacers in the compounds of the invention (for a review, see Simons, Spacers, probability, and yields, Bioconjug Chem 1999 Jan-Feb;10(1):3-8). Some non-limiting examples of sequences that have been used in sFvs include EGKSSGSGSESKEF (SEQ ID NO: 10), one or more copies of GGGGS [also known as (G₄S)_x (SEQ ID NO: 46)] (Newton *et al.*, Angiogenin single-chain immunofusions: influence of peptide linkers and spacers between fusion protein domains, Biochemistry 1996 Jan 16;35(2):545-53), GSGS [also known as (GSGS)_x (SEQ ID NO: 47)] and GSSG [also known as (GSSG)_x (SEQ ID NO: 48)].

- Please delete paragraph [0211] and replace it with the following paragraph:

[0211] The PCR product from the overlap PCR was gel purified and cloned directly into the mammalian expression vector pcDNA3.1D/V5-His-TOPO® expression vector (Invitrogen, Carlsbad, CA). This expression vector includes a CMV-derived promoter for high-level, constitutive expression; a C-terminal V5 epitope tag that can be detected with anti-V5 antibody; and a further C-terminal 6xHis tag (SEQ ID NO: 49) that can be detected with an anti-6xHis tag (SEQ ID NO: 49) antibody or used to purify the IL-2-5A fusion protein. Anti-V5 and anti-6xHis (SEQ ID NO: 49) antibodies are available from Invitrogen.

- Please delete paragraph [0217] and replace it with the following paragraph:

[0217] IMAC chromatography was used to purify IL-2-sFv fusion protein from transiently transfected cells. In brief, about 400 ml of media from transfected COS-1 cells incubated for 48 to 144 hours was harvested. The media was pooled and Imidazole was added to a final concentration of 10 mM. A Pellicon cassette System (Millipore Bioscience, Bedford, MA) was used to concentrate the pool to a final volume of ~75 ml. The concentrated sample was then purified using a nickel column, to which the 6xHis tag (SEQ ID NO: 49) binds.

- Please delete paragraph [0219] and replace it with the following paragraph:

[0219] A Carboxy terminal fusion of IL-2 with a pIgR-directed sFv designed to favor dimeric sFv formation was constructed by cloning IL-2 without its signal peptide into the AvrII site of the sFv depicted in Fig. 5. A linker comprising of (Gly₃ Ser)₂ (SEQ ID NO: 50) was included in the 5' oligonucleotides and two Stop codons were included in the 3' oligonucleotides.

- Please delete paragraph [0235] and replace it with the following paragraph:

[0235] An amino terminal fusion of IL-2 with an sFv designed to favor sFv dimer formation was constructed by cloning IL-2, with its signal peptide, into the Nhe1 site of the sFv shown in Fig. 5. A linker consisting of (Gly₂Ser)₂ (SEQ ID NO: 51) had previously been ligated to the 5' end of this sFv.

- Please delete paragraph [0237] and replace it with the following paragraph:

[0237] Twenty five cycles of PCR were performed at 58°C. The PCR product was cloned into an intermediate vector: pCR-BluntII-TOPO (Invitrogen, Carlsbad, CA). The IL-2 PCR product was cut out from this intermediate vector using EcoRV and Nhe1, gel purified and

cloned into the Nhe1 site of (Gly₂Ser)₂ (**SEQ ID NO: 51**)-sFv in the mammalian expression vector pDIZ. pDIZ was constructed as follows: A 4882bp Spe1/EcoRV fragment was isolated from pcDNA 3.1 Hygro (Invitrogen, CA) and ligated to a Spe1/Xmn1 fragment from gWiz (Gene Therapy Systems Inc.). A plasmid map of pDIZ is shown in Fig. 7.

- Please delete paragraph [0248] and replace it with the following paragraph:

[0248] The forward primer used to generate the α -IFN 544 bp PCR product was designed to include sequences encoding a synthetic linker encoding 5 amino acids (Gly-Gly-Gly-Gly-Ser) (**SEQ ID NO: 46**) that are connected in frame to the C-terminus sFv polypeptide. The 3-step PCR amplification reaction included 5 cycles with annealing temperature at 55°C followed by 30 cycles at 60°C. The 544 bp PCR product was gel purified and cloned into the pCR Blunt II TOPO intermediate vector. Miniprep DNA was made and positives clones verified for the PCR product by DNA sequencing. Following sequence confirmation, the PCR product was excised by digesting the maxiprep DNA with AvrII and SalI restriction enzymes, then ligated into AvrII / SalI digested APL-10 pELK vector DNA using T4 DNA ligase. Miniprep DNA was prepared and positive clones confirmed by DNA sequencing. Positive vector clones are illustrated in Figure 1 and contain the chimeric DNA sequence (SEQ ID NO: 18) which encodes a chimeric protein containing the following protein domain structural orientation: (NH₂)-pel-B leader-sFv-Gly₄Ser linker- α -IFN -(COOH). **Linker shown in SEQ ID NO: 46.**

- Please delete paragraph [0256] and replace it with the following paragraph:

[0256] The forward primer used to generate the partial APL-10- \square -IFN 551 bp PCR product was designed to include sequences encoding a synthetic linker encoding 5 amino acids (Gly-Gly-Gly-Gly-Ser) (**SEQ ID NO: 46**) that can be inserted in frame to the C-terminus of sFv polypeptide APL-10. The 3-step PCR amplification reaction included 5

cycles with annealing temperature at 55°C followed by 30 cycles at 60°C. The 551 bp PCR product was QIAquick column purified and cloned into the pCR Blunt II TOPO intermediate vector. Miniprep DNA was made and positives clones verified by DNA sequencing. Following sequence confirmation, the PCR product was inserted into the AvrII / SalI sites of APL-10E vector (pELK vector derivative), or AvrII / XhoI digested APL-2005S vector (pSyn vector derivative) DNAs. Miniprep DNA was prepared and positive clones confirmed by DNA sequencing. Positive vector clones are illustrated in Figure 2 and contain the chimeric DNA sequence (SEQ ID NO: 24) which encode for a chimeric protein containing the following domains and oriented from the N-terminus: (NH₂)-pel-B leader-sFv-Gly₄Ser linker-β-IFN-(COOH). **Linker shown in SEQ ID NO: 46.**

sFv-β-IFN chimera DNA sequence (SEQ ID NO: 24):

ATGAAATACC	TATTGCCTAC	GGCAGCCGCT	GGATTGTTAT	TACTCGCGGC	CCAGCCGGCC	60
ATGGCC <u>CAGG</u>	TGCAGCTGCA	GCAATCAGGG	GGAGGGTGG	TCCAGCCTGG	GAGGTCCCTG	120
AGACTCTCCT	GTGCAGCCTC	TGGATTCAACC	TTCAGTAGCT	ATGCTATGCA	CTGGGTCCGC	180
CAGGCTCCAG	GGAAGGGGCT	GGAGTGGTGC	TCAGCTATTA	GTGGTAGTGG	TGGTAGCACA	240
TACTACGCAG	ACTCCGTGAA	GGGCCGGTTC	ACCATCTCCA	GAGACAAACGC	CAAGAACTCA	300
CTGTATCTGC	AAATGAACAG	CCTGAGAGCC	GAGGACACGG	CTGTGTATTA	CTGTGCGAGA	360
GATACCCGAG	GGTACTTCGA	TCTCTGGGC	CGTGGCACCC	TGGTCACCGT	CTCCTCAGGT	420
GGCGGAGGGT	CATCTGAGCT	GAUTCAGGAC	CCTGCTATGT	CTGTGGCCTT	GGGACAGACA	480
GTCAGAATCA	CATGTCAAGG	GGACAGTCTC	AGAAAGTATC	ATGCAAGCTG	GTATCAGCAG	540
AAGCCAGGGC	AGGCCCTGT	TCTTGTACATC	TATGGTAAGA	ATGAACGTCC	CTCAGGGATC	600
CCAGAGCGAT	TCTCTGGTC	CACCTCAGGA	GACACAGCTT	CCTTGACCAT	CAGTGGCTC	660
CAGGCGGAAG	ATGAGGCTGA	CTATTACTGT	CACTCCGAG	ACTCTAATGC	TGATCTTGTG	720
GTGTTCGCG	GAGGGACCAA	GGTCACCGTC	CTAGGTGGT	CGGGAGGGTC	AATGAGCTAC	780
AACTTGCTTG	GATTCTACA	AAGAACAGC	AATTTCACT	GTCAGAAAGCT	CCTGTGGCAA	840
TTGAATGGGA	GGCTTGAATA	CTGCCTCAAG	GACAGGATGA	ACTTGACAT	CCCTGAGGAG	900
ATTAAGCAGC	TGCAGCAGTT	CCAGAAGGAG	GACGCCGCAT	TGACCATCTA	TGAGATGCTC	960
CAGAACATCT	TTGCTATTT	CAGACAAGAT	TCATCTAGCA	CTGGCTGGAA	TGAGACTATT	1020
GTTGAGAACCC	TCCTGGCTAA	TGTCTATCAT	CAGATAAAC	ATCTGAAGAC	AGTCCTGGAA	1080
GAAAAAACTGG	AGAAAGAAGA	TTTCACCAGG	GGAAAACCTCA	TGAGCAGTCT	GCACCTGAAA	1140
AGATATTATG	GGAGGATTCT	GCATTACCTG	AAGGCCAAGG	AGTACAGTCA	CTGTGCCTGG	1200

ACCATAGTCA GAGTGGAAAT CCTAAGGAAC TTTTACTTCA TTAACAGACT TACAGGTTAC 1260
CTCCGAAACT AA 1272

- Please delete paragraph [0258] and replace it with the following paragraph:

[0258] The IFN β region was amplified by PCR using primers MG sigP(-)HIFN β 5' (SEQ ID NO:27) and MG HIFN β 3' (SEQ ID NO:28) and pDIZ HIFN β -APL10 as a template. The wild-type signal peptide was removed and replaced with a (Gly-Gly-Gly-Ser)x2 linker (SEQ ID NO: 50). The signal peptide minus HIFN β PCR product was digested with AvrII and NotI and inserted into pgWIZtpaSigP-APL10 cut with the same enzymes to make pgWIZtpaSigP-APL10-HIFN β . The resulting products were screened by miniprep and verified by sequencing. To subclone the tpaSigP-APL10-HIFN β into pDIZ, pgWIZtpaSigP-APL10-HIFN β was cut with EcoRV and NotI and the tpaSigP-APL10-HIFN β fragment was gel purified.

MG sigP(-)HIFN β 5' (SEQ ID NO:27):

5'- GTCCTAGGTGGCGGCGGAAGCGGGGGAGGCTCCATGAGCTACAACTTGCTTGGATTCCCTAC
AAAGAAGCAGCA -3'

MG HIFN β 3' (SEQ ID NO:28)

5'- TGCAGGCCGCTTAGCTAGCTTATTAGTTGGAGGTAACCTGTAAGTCTGTTAATGAAGTAA
AAGTTCCCT -3'

The tpaSigP-APL10-HIFN β fragment was inserted into pDIZ cut with EcoRV and NotI to make pDIZtpaSigP-APL10-HIFN β . The full-length insert was sequenced and verified to be correct.

TPA SigP-APL10-IFN β (SEQ ID NO: 29):

ATGGATGCAA	TGAAGAGAGG	GCTCTGCTGT	GTGCTGCTGC	TGTGTGGAGC	AGTCTTCGTT	50	<u>60</u>
TCGCCAGCC	AGGTACAGCT	GCAGCAATCA	GGGGGAGGCG	TGGTCCAGCC	TGGGAGGTCC	100	<u>120</u>
CTGAGACTCT	CCTGTGCAGC	CTCTGGATT	ACCTTCAGTA	GCTATGCTAT	GCACTGGTC	150	<u>180</u>
CGCCAGGCTC	CAGGGAAGGG	GCTGGAGTGG	GTCTCAGCTA	TTAGTGGTAG	TGGTGGTAGC	200	<u>240</u>
ACATACTACG	CAGACTCCGT	GAAGGGCCGG	TTCACCATCT	CCAGAGACAA	CGCCAAGAAC	250	<u>300</u>
TCACTGTATC	TGCAAATGAA	CAGCCTGAGA	GCCGAGGACA	CGGCTGTGTA	TTACTGTGCG	300	<u>360</u>
AGAGATAACCC	GAGGGTACTT	CGATCTCTGG	GGCCGTGGCA	CCCTGGTCAC	CGTCTCCTCA	350	<u>420</u>
GGTGGCGGAG	GGTCATCTGA	GCTGACTCAG	GACCCTGCTA	TGTCTGTGGC	CTTGGGACAG	400	<u>480</u>
ACAGTCAGAA	TCACATGTCA	AGGGGACAGT	CTCAGAAAGT	ATCATGCAAG	CTGGTATCAG	450	<u>540</u>
CAGAAGCCAG	GGCAGGCCCC	TGTTCTTGT	ATCTATGGTA	AGAATGAACG	TCCCTCAGGG	500	<u>600</u>
ATCCCAGAGC	GATTCTCTGG	GTCCACCTCA	GGAGACACAG	CTTCCTTGAC	CATCAGTGGG	550	<u>660</u>
CTCCAGGCGG	AAGATGAGGC	TGACTATTAC	TGTCACTCCC	GAGACTCTAA	TGCTGATCTT	600	<u>720</u>
GTGGTGTTCG	CGGGAGGGAC	CAAGGTCAACC	GTCCTAGGTG	CGGGCGGAAG	CGGCGGAGGC	650	<u>780</u>
TCCATGAGCT	ACAACTTGCT	TGGATTCTCA	CAAAGAAGCA	GCAATTTC	GTGTCAGAAC	700	<u>840</u>
CTCCTGTGGC	AATTGAATGG	GAGGCTTGAA	TAUTGCCTCA	AGGACAGGAT	GAACTTTGAC	750	<u>900</u>
ATCCCTGAGG	AGATTAAGCA	GCTGCAGCAG	TTCCAGAAGG	AGGACGCCGC	ATTGACCATC	800	<u>960</u>
TATGAGATGC	TCCAGAACAT	CTTGCTATT	TTCAGACAAG	ATTCACTAG	CACTGGCTGG	850	<u>1020</u>
AATGAGACTA	TTGTTGAGAA	CCTCCTGGCT	AATGTCTATC	ATCAGATAAA	CCATCTGAAG	900	<u>1080</u>
ACAGTCCTGG	AAGAAAAACT	GGAGAAAGAA	GATTTCACCA	GGGGAAAAGT	CATGAGCAGT	950	<u>1140</u>
CTGCACCTGA	AAAGATATTA	TGGGAGGATT	CTGCATTACC	TGAAGGCCAA	GGAGTACAGT	1000	<u>1200</u>
CACTGTGCCT	GGACCATAGT	CAGAGTGGAA	ATCCTAAGGA	ACTTTACTT	CATTAACAGA	1050	<u>1260</u>
CTTACAGGTT	ACCTCCGAAA	CTAA	4074-1284				

- Please delete paragraph [0268] and replace it with the following paragraph:

[0268] Figure 1 shows the schematic structure of the sFv directed to a pIgR epitope used for the following *in vivo* transport studies. Indicated are the Pelb leader (a leader sequence that directs secretion from *E. coli*); linker (amino acid sequence (gly-gly-gly-gly-ser)_n) (**SEQ ID NO: 46**); H₆, (6xHis tag) (**SEQ ID NO: 49**); cysteine tag (amino acid sequence gly-gly-gly-gly-cys) (**SEQ ID NO: 52**); and the heavy and light chains of the sFv. The selected sFv comprises an altered FR2 region, an internal unpaired cysteine, a C-terminal His tag, and a single linker repeat. This construct directs near homogenous dimeric sFv formation.

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- Between pages 88-89, on a separate page, please insert the Sequence Listing submitted herewith.